Intra-uterine growth restriction and the programming of left ventricular remodelling in female rats

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Epidemiological studies link intra-uterine growth restriction (IUGR) with increased incidence of hypertension and cardiac disease in adulthood. Our rat model of IUGR supports this contention and provides evidence for the programming of susceptibility for hypertension in all offspring. Moreover, in the female offspring only, gross anatomical changes (cardiac ventricle to body ratios) and increased left cardiac ventricular atrial natriuretic peptide (ANP) mRNA levels provide evidence for programming of cardiac disease in this gender. The aim of the current study was to measure changes in cardiac tissue that support remodelling that could be implicated in the initiation of hypertrophy. Adult female rats from our IUGR model and age- and sex-matched controls were killed at 12 weeks of age. Left cardiac ventricles were removed and used for monitoring changes in several key genes, Na^+,K^+-ATPase β1 protein expression, cardiomyocyte morphology and contractility as well as citrate synthase and aconitase activities. When compared to controls, female offspring of our IUGR rat model exhibit higher expression (mRNA) of ANP and the atrial isoform of the myosin light chain, lower levels of Na^+,K^+-ATPase β1 protein, increased cardiomyocyte depth and volume, increased sarcomere length, diminished cardiomyocyte contractility and lower aconitase activity. Female offspring of our IUGR rat model exhibit changes as adults that are consistent with the onset of cardiac remodelling. The decrease in aconitase activity suggests that oxidative stress may be implicated in this response.

(Resubmitted 16 February 2005; accepted after revision 14 March 2005; first published online 17 March 2005)
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Epidemiological studies suggest that adverse intra-uterine environments during fetal life are associated with a higher incidence of hypertension, type II diabetes and coronary disease (Barker *et al.* 1993). Animal models of intra-uterine growth restriction (IUGR) support this contention and are invaluable for studying underlying mechanisms in the programming of these pathologies.

In our laboratory, we developed a model of IUGR induced by a low-sodium diet given to pregnant rats over the last week of gestation (Roy-Clavel *et al.* 1999). The offspring of IUGR model rats exhibit higher systolic blood pressure, changes in the renin–angiotensin–aldosterone system and renal dysfunction as adults (Battista *et al.* 2002). Furthermore, left cardiac ventricle to total body weight ratios are elevated in female but not in male offspring of IUGR rats when they reach adulthood (Battista *et al.* 2002). In adult females, but not males, levels of atrial natriuretic peptide (ANP) messenger RNA were increased in the cardiac left ventricle compared to controls. Although the increases of cardiac mass and ANP mRNA levels suggest an increase in cardiomyocyte size, cell sizes were not reported nor were other markers that usually accompany hypertrophy (Battista *et al.* 2002). Thus, it is now important to determine the extent to which myocardial remodelling is based upon changes in the cardiomyocytes of female rats.

Ventricular hypertrophy is a well-established risk factor for cardiovascular mortality in humans. Cardiac hypertrophy is defined as the increase in myocardial mass, often induced in response to increased wall tension (James *et al.* 2000). In the absence of a proliferative potential, the heart reduces wall stress by increasing individual cell volume (hypertrophy). Maladaptive hypertrophy leading to disease is associated with distinct morphological, molecular and functional changes. In this regard, increased cardiomyocyte size in pressure-overload is accompanied

 DOI: 10.1113/jphysiol.2004.078139

by changes in the expression and activity of several ion channels and transporters (Tomaselli & Marban, 1999; Swynghedauw, 1999) as well as the reactivation of a fetal gene programme (Chien *et al.* 1991; Nakao *et al.* 1992; Gupta & Gupta, 1997). Some of these genes code for contractile proteins such as atrial myosin light chain (aMLC-1) and myosin heavy chain (MHC) (Schaub *et al.* 1998; Diffee & Nagle, 2003), others, such as ANP, regulate blood pressure by controlling Na^+ and water homeostasis. Reactivation of these genes is considered a hallmark for cardiac hypertrophy. Furthermore, their modified expression in hypertrophy may underlie alterations in myocyte function and contractility.

Cardiac hypertrophy is also associated with oxidative stress and the production of reactive oxygen species (ROS) (Byrne *et al.* 2003). For example, in a murine model of pressure-overload, cardiac hypertrophy was noted simultaneously with increased heme oxygenase-1 mRNA expression and lipid peroxidation, both signs of oxidative stress (Date *et al.* 2002). This hypertrophic response was reversed by the administration of the ROS scavenger, *N*-2-mercaptopropionyl glycine. Furthermore, in an *in vitro* model of cardiac hypertrophy, ROS production was observed concomitant with a decrease in the activity of the tricarboxylic acid (TCA) cycle enzyme, aconitase (Nakagami *et al.* 2003). As aconitase is extremely sensitive to ROS (Hausladen & Fridovich, 1994), its suppression may serve as a useful marker for oxidative stress and cardiac hypertrophy.

In view of the above, the aim of the present study was to characterize changes that occur in left cardiac ventricles of the adult female offspring of our IUGR rat model. The males were not studied because previous data clearly showed that there is no sign of initiation of hypertrophy in this gender. We propose that alterations in cardiomyocyte size, gene expression, contractility and the activities of some enzymes of the TCA cycle may be noted. These changes would confirm that altered fetal environment could lead to the remodelling of cardiomyocytes and that this model would help elucidate underlying mechanisms implicated in the hypertrophic response.

Methods

Animals, experimental design, diet and housing

Experimental procedures were reviewed and approved by the local Animal Care Committee that is accredited by the Canadian Council on Animal Care. Female Sprague-Dawley rats (Charles River Canada, St-Constant, Québec, Canada) were obtained, bred and housed as detailed previously (Roy-Clavel *et al.* 1999). Control rats ($n = 30$) were the female offspring of dams maintained on a normal diet containing 0.2% sodium (basal diet 5755; PMI Feed, Ren's Feed and Supplies, Oakville, Ontario, Canada) and tap water. The IUGR females ($n = 30$) were the offspring of dams placed on a 0.03% sodium diet (low-sodium diet 5881; PMI Feed) and demineralized water for the last week of gestation. After birth, all offspring received normal rat chow (0.2% sodium) and tap water. At 12 weeks of age, females were killed by decapitation (at 9.00–9.30 h), body weights recorded and hearts removed. Atria were trimmed away and left cardiac ventricles used. For molecular analyses and enzyme activity measures, tissues were immediately frozen in liquid nitrogen and stored at -80°C until further use. For cardiomyocyte morphology and contractility studies, hearts were processed immediately.

Total RNA isolation

Total RNA from left cardiac ventricles (100 mg) was extracted using TRIzol reagent (Invitrogen Canada Inc., Burlington, Ontario, Canada). For the DNA microarray, pooling of tissue was done to reduce inter-individual variation and to better characterize the population (IUGR *versus* control). Left cardiac ventricles of five animals from different litters were pooled, reduced to powder for RNA extraction, and used for DNA microarray. To confirm the results obtained from the DNA microarray, seven to nine left cardiac ventricles from animals from different litters were reduced separately for RNA extraction.

Gene expression microarray

Gene expression quantification was performed as described by Vasseur *et al.* (2003). Briefly, 20 mg total RNA was converted to cDNA with SuperScript reverse transcriptase (Gibco-BRL, Invitrogen), using T7-oligo-d(T)24 as a primer. Second-strand synthesis was performed using T4 DNA polymerase and *E. Coli* DNA ligase followed by blunt ending by T4 polynucleotide kinase. cDNA was isolated by phenol-chloroform extraction using phase lock gels (Brinkmann). cDNA was *in vitro* transcribed using the T7 BioArray High Yield RNA Transcript Labelling Kit (Enzo Biochem, New York, NY, USA) to produce biotinylated cRNA. Labelled cRNA was isolated using an RNeasy Mini Kit column (Qiagen). Purified cRNA was fragmented to 200–300 mers. Fragmented cRNA (15 mg) was hybridized to a U34A Genechip (Affymetrix, Santa Clara, CA, USA). Microarrays were processed in an Affymetrix GeneChip Fluidic Station 400. Results were analysed as previously described (Sasik *et al.* 2002). Gene expression quantification microarray experiments were repeated twice using RNA from two sets of five animals. Genes were considered to be present at 95% confidence ($P < 0.05$) for their expression, and differentially expressed when at least a two-fold (arbitrary cut-off) difference between control and experimental values was evident.

Table 1. PCR primers designed (by Alpha DNA, Montréal, Québec, Canada) for genes of interest

Gene		Primers	Product size (bp)
Atrial myosin light chain-1 (aMLC-1)	FP	CCAGCCTGAAGAGATGAAT	376
	RP	CCAGTATGAGTCCAGTGCTC	
Atrial natriuretic peptide (ANP)	FP	TTCAAGAACCTGCTAGACCA	302
	RP	GCTCCAATCCTGTCAATCCT	
β-Myosin heavy chain (β-MHC)	FP	GGCCTGAATGAAGAGTAGAT	98
	RP	GTGTTTCTGCCTAAGGTGCT	
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	FP	GGTGATGCTGGTGCTGAGTA	369
	RP	GGATGCAGGGATGATGTTCT	
Myosin regulatory light chain-2 (MRLC-2)	FP	GGAGGAGACCATTCTCAACG	110
	RP	TGGAGAACCTCTCTGCTTGC	
Na^+,K^+-ATPase β1	FP	TGGAGACTTACCCTCTGACGATG	357
	RP	GGCTAGTGGGAAAGATTTGTGC	

FP: forward primer (5′→3′); RP: reverse primer (5′→3′).

Reverse transcription polymerase chain reaction

Verification of results from gene expression microarray was done by RT-PCR for five selected genes (aMLC-1, ANP, β-MHC, myosin regulatory light chain-2 (MRLC-2) and for the β1 subunit of the Na^+,K^+-ATPase pump (Na^+,K^+-ATPase β-1)) using the same pooled samples. Results were reported using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as internal control. For confirmation of these results, the procedure was carried out on triplicate samples from individual animals as well. PCR primers specific for genes of interest were designed with PRIMER3 (www.genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi) using sequence data from National Center for Biotechnology Information (NCBI) database (Table 1).

Single-stranded cDNA was created by reverse transcription (SuperScriptTM II RNase H- Reverse Transcriptase, Invitrogen) and PCR (Taq DNA Polymerase, Invitrogen) performed according to the procedure detailed by the manufacturer. The annealing step was carried out for 1 min at temperatures determined for each gene (aMLC-1, 53°C; ANP, 50°C; β-MHC, 51°C; MRLC-2, 56°C; Na^+,K^+-ATPase β-1, 53°C; and GAPDH, 51°C). The extension step was performed at 72°C for 1 min. PCR results were taken during the exponential phase. The number of cycles needed for amplification was determined for each gene (GAPDH, 20; β-MHC, 21; Na^+,K^+-ATPase β1, 22; MRLC-2, 24; aMLC-1, 27; ANP, 27). PCR products were electrophoresed on 1% agarose gels containing ethidium bromide. Amplification products were quantified using Alpha Imager software (Alpha Innotech Corporation, San Leandro, CA, USA).

Western blotting for Na^+,K^+-ATPase β1

Frozen powder (200 mg) from each left cardiac ventricle (five animals per group) was homogenized in Tris buffer (pH 7.4) and centrifuged at 2500 *g* for 10 min. Supernatants collected were then centrifuged at 300 000 *g* for 1 h and pellets resuspended in sample buffer. Proteins (20 μg lane^{-1}) were separated on 15% SDS-polyacrylamide gels and transferred to nitrocellulose membranes (NitroPure, Osmonics Inc., Gloucester, MA, USA). Membranes were blocked with Tris buffer saline (TBS; 150 mM NaCl, 10 mM Tris-HCl, pH 7.5)−0.1% Tween 20–5% milk and then incubated with mouse anti-Na^+,K^+-ATPase β1 subunit antibodies (Upstate Biotechnology, Waltham, MA, USA) 1 : 1000 overnight at 4°C, washed, and then incubated with horseradish peroxidase-conjugated goat anti-mouse immunoglobulin (Upstate Biotechnology) 1 : 10 000 for 30 min at room temperature (22°C). Equal protein loading was determined on duplicate gels using mouse anti-β-actin primary antibodies (Abcam, Cambridge, MA, USA). Immunoreactive bands were visualized with the enhanced ECLTM chemoluminescence detection system (Amersham, Piscataway, NJ, USA).

Cardiomyocyte morphology: cardiomyocyte isolation, morphometric analyses and contractility measures

Left ventricular myocytes were isolated early in the morning following retrograde perfusion of the heart with proteolytic enzymes as previously detailed (Chorvatova & Hussain, 2003). Briefly, the heart was removed and cleared of blood by retrograde perfusion for 1–2 min with Hepes-buffered physiological salt solution containing 0.75 mmol l^{-1} $CaCl_2$. This was followed by 5 min perfusion with Ca^{2+}-free Hepes-buffered physiological salt solution containing 0.1 mmol l^{-1} EGTA and then with the enzyme solution containing 1.0 mg ml^{-1} collagenase (type II; Worthington, Lakewood, NJ, USA) and 0.03 mg ml^{-1} protease (type XXIV; Sigma-Aldrich, Oakville, Ontario, Canada) in low $CaCl_2$ (50 μmol l^{-1}). The left ventricle was dissected free, sliced into

Table 2. Relative changes in expression of hypertrophic marker genes in female adult IUGR rats

		Microarray	
Gene description	Probe set name	1st	2nd
Atrial myosin light chain-1	X51531cds_at	+6.37	+2.24
Natriuretic peptide precursor A	X01118_at	+4.02	1.30
Rat cardionatrin precursor	E00775cds_s_at	+3.58	1.21
Rat atrial natriuretic factor (ANF)	E00903cds_s_at	+3.26	0.93
Rat γ atrium natriuretic polypeptide γ-rANP	E00698cds_s_at	+3.23	1.09
Rat monoamine oxidase A gene	D00688_s_at	+2.14	1.69
Integrin, α1	X52140_at	1.25	+3.48
Butyrate response factor 1	rc_AI136891_at	1.05	+2.20
Integrin, β1	rc_AI177366_at	−2.09	0.89
Na^+,K^+-ATPase transporting β1 polypeptide	rc_AI230614_s_at	−2.45	1.25
Myosin regulatory light chain	D14688_s_at	0.96	1.35
Myosin heavy chain, cardiac muscle, fetal	X15939_f_at	1.12	1.65

Results are expressed as *n*-fold change in expression in IUGR females when compared to control rats.

smaller chunks and agitated in the enzyme solution supplemented with 1.0% bovine serum albumin (Sigma). Aliquots of myocytes were harvested at 5-min intervals by filtration of the digest through 250 μm monofilament nylon cloth followed by gentle centrifugation of the filtrate (40 *g* for 30 s). The pellets of myocytes were re-suspended in enzyme-free isolation solution containing 0.75 mmol l^{-1} $CaCl_2$ and maintained in a Petri dish at 4°C until used. The process was repeated two to three times to obtain a total of at least three batches of myocytes from each heart. Myocyte isolation was carried out at 36 ± 1°C.

Spatial and volumetric distribution of unstained single cardiomyocytes was determined (wavelength (λ) of excitation/emission = 488/520 nm) using a scanning confocal microscope (Zeiss LSM 510, Thornwood, NY, USA) equipped with a PlanNeofluor 63 ×/1.3 oil immersion objective. Three-dimensional confocal images were recorded using 2.0-μm steps in *z*-stack. Cell length (l), width (w) and depth (d) were determined from these images and cell volume (V) and cross-sectional area (CSA) calculated, using the equation for an elliptic cylinder: $V = l \times w \times d \times \pi/4$ and $\mathrm{CSA} = w \times d \times \pi/4$, respectively. Sarcomere length was measured in unloaded cells and was determined from transmission images. Sarcomere number was calculated in each cell as a ratio between cell length and sarcomere length. Cardiomyocyte shortening was studied by line scan confocal imaging of single cardiomyocytes perfused extracellularly with solutions heated to 35 ± 1°C (Chorvatova & Hussain, 2003). Cells were field stimulated at 0.5 Hz by a constant-voltage isolated stimulator (DS-A2, Digitimer Ltd, Harvard, St-Laurent, Québec) through a trigger generator (DG-2, Digitimer Ltd) using platinum electrodes incorporated into a homemade bath. Contractility was measured in isolated single cardiomyocytes as percentage change in the cell length, defined as (maximal cell length–shortening)/maximal cell length.

Enzyme assays

Frozen powdered tissue (30 mg) was homogenized in 0.1 mM Tris-HCl/15 mM tricarballylic acid solution (pH 7.8), centrifuged at 9500 *g* for 10 min at 4°C and the supernatant collected was immediately processed for enzyme determinations. Citrate synthase activity was measured at 412 nm using 5,5′–dithiobis-2-nitrobenzoic acid as substrate (Nulton-Persson & Szweda, 2001). Aconitase activity was determined at 340 nm by the formation of NADPH following α-ketoglutarate production from isocitrate (Comte *et al.* 2002). Protein concentrations were determined with the Bio-Rad Assay kit (Bio-Rad, Mississauga, Ontario, Canada) using bovine serum albumin as standard. Enzyme activities are expressed in units (U) per mg of protein, where U is defined as the amount of enzyme catalysing the conversion of 1 μmol of substrate per min at 37°C. Activities for each enzyme were determined for six animals per group.

Statistical analysis

Results are expressed as mean ± S.E.M. and compared using Student's *t* test. Significance assumed at $P < 0.05$.

Results

Gene expression microarray

Those genes whose expression was altered by at least 2-fold in the IUGR group when compared to controls are listed (Table 2). In the first assay, expression of aMLC-1, of four genes related to ANP and of monoamine oxidase A were higher, whereas, expression of integrin β-1 and

Na^+,K^+-ATPase $\beta 1$ genes were lower in IUGR rats when compared to controls. In the second assay, expression of aMLC-1, integrin α-1 and butyrate response factor 1 increased in the IUGR rats by at least 2-fold when compared to controls.

RT-PCR validation

RT-PCR validation of aMLC-1, ANP, β-MHC, MRLC-2 and Na^+,K^+-ATPase $\beta 1$ was done on pooled tissue samples prepared and used for gene expression microarray assays. The results obtained from the two techniques were similar (data not shown).

Gene expression microarray methodology was used to screen for gene products that may be implicated in cardiac hypertrophy. In view of the slight discrepancy between our two microarray assays, distribution of mRNA levels between the two populations of animals was examined using mRNAs from individual animals. Although MRLC-2 (Fig. 1*A*), β-MHC (Fig. 1*B*) and Na^+,K^+-ATPase $\beta 1$

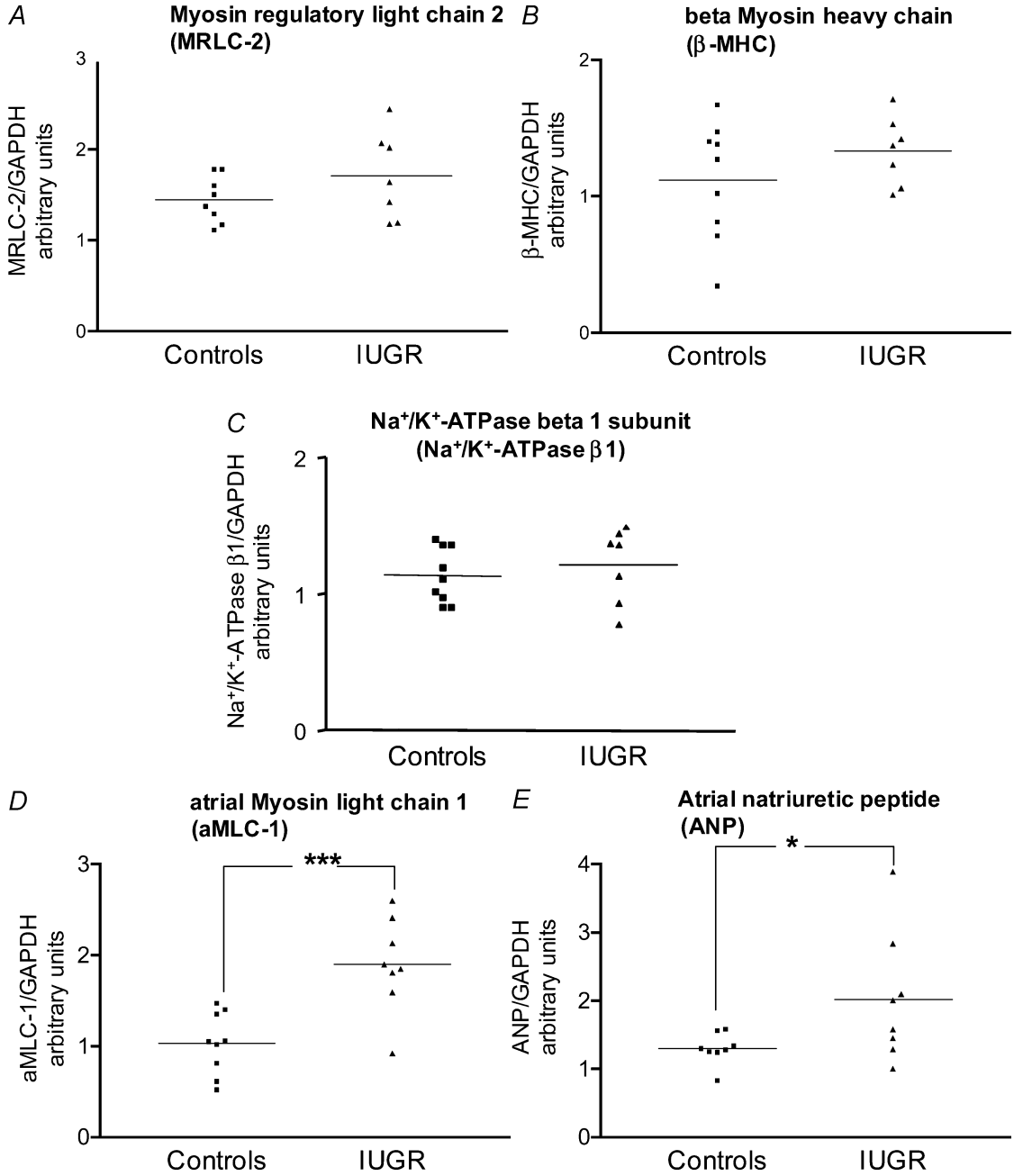

Figure 1. Comparative gene expression
Comparative gene expression for MRLC-2 (*A*), β-MHC (*B*), Na^+,K^+-ATPase $\beta 1$ (*C*), aMLC-1 (*D*) and ANP (*E*) relative to GAPDH by RT-PCR in left ventricles from adult female control and IUGR rats using column statistic analysis. $^*P \leq 0.05$; $^{***}P \leq 0.001$ compared to controls.

(Fig. 1*C*) mRNA levels are not different between the two groups of animals, expression of aMLC-1 (Fig. 1*D*) and ANP (Fig. 1*E*) mRNA increased in the left ventricles of our IUGR adult females.

Na^+,K^+-ATPase β1 Western blotting analysis

A representative Western blot (Fig. 2*A*) shows that the intensities of the Na^+,K^+-ATPase β1 immunoreactive bands are lower in samples from left ventricles of IUGR females when compared to those from controls. From densitometric analyses, expression of Na^+,K^+-ATPase β1 protein (expressed in arbitrary units) is 2-fold lower in IUGR females when compared to controls (Fig. 2*B*). Mean optical density values for Na^+,K^+-ATPase β1 protein are 0.56 for IUGR females and 1.23 for controls.

Cardiomyocyte morphology and contractility

Cardiomyocytes isolated from left ventricles of the IUGR females were significantly greater ($P < 0.05$) in depth than those from control females (Table 3). We also observed significant increase of 23% ($P < 0.05$) in cell volume (Table 3). Cross-sectional area of single myocytes was also significantly increased (Table 3). In addition, sarcomere length was also significantly greater ($P < 0.05$) in cardiomyocytes from the IUGR females when compared to those of controls (Table 3). In contrast, no significant differences were found in cardiomyocyte length and width as well as mean sarcomere number per cell amongst the two groups of animals. Finally, cell contractility (0.5 Hz, $35 \pm 1^{\circ}$C) was reduced by 29% in IUGR females when compared to controls.

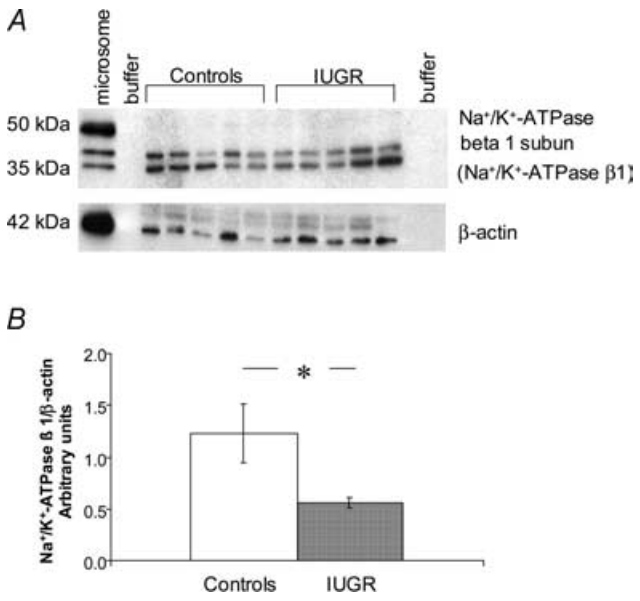

Figure 2. Western blot analysis of Na^+,K^+-ATPase β1 protein from left ventricles of adult female control and IUGR rats
Representative immunoblot (*A*) and corresponding densitometric analyses (*B*). Brain microsomes serve as positive control and buffer as negative control. $^*P \leq 0.05$ compared to controls.

Table 3. Morphometric and contractility measurements for isolated cardiomyocytes from adult female control and IUGR rats

Cell	Controls	IUGR
Length (μm)	121.7 ± 3.4 (22)	125.7 ± 2.9 (36)
Width (μm)	30.3 ± 1.7 (22)	30.1 ± 1.0 (36)
Depth (μm)	16.2 ± 0.5 (22)	$19.4 \pm 0.7^{**}$ (36)
Volume (pl)	45.7 ± 2.6 (22)	$56.0 \pm 2.9^{**}$ (36)
Cross-sectional area (μm)	380 ± 23 (22)	$442 \pm 19^{*}$ (36)
Sarcomeric length (μm)	1.75 ± 0.01 (22)	$1.78 \pm 0.01^{*}$ (36)
Sarcomeric number	75.5 ± 2.3 (22)	74.1 ± 1.8 (36)
Change in cell length (%)	6.2 ± 0.5 (15)	$4.4 \pm 0.4^{**}$ (32)

$^*P \leq 0.05$, $^{**}P \leq 0.01$ *versus* controls; number of cells analysed shown in parentheses.

Tricarboxylic acid cycle enzyme activities

Activities of citrate synthase were found not to differ amongst the two groups of animals (Fig. 3*A*). In contrast, activities of the second mitochondrial enzyme, aconitase, were significantly lower ($P < 0.01$) in samples from left cardiac ventricles of IUGR females when compared to those of controls (Fig. 3*B*).

Discussion

The increase in left cardiac ventricle to total body weight ratios and in cardiac ventricle ANP mRNA levels in our female IUGR rats is suggestive of hypertrophy (Battista *et al.* 2002). However, morphological evidence for early cardiomyocyte hypertrophy has been found in our present study using scanning confocal microscopy. In this regard, increased cell depth accounts for the increase in cardiomyocyte volume in our IUGR females while a significantly greater cross-sectional area indicates a thicker left ventricular wall. These data correlate with the increase in left ventricle to body weight ratio, identified previously in this model. Moreover, these changes in volume, via increased cross-sectional area, appear to occur during the period of compensated hypertrophy (Onodera *et al.* 1998). Myocyte lengthening is noted more so during the progression to heart failure as is the excessive addition of sarcomeres in series (Onodera *et al.* 1998). We do report an increase in sarcomere length in the IUGR females that may indicate either the initiation of the formation of series sarcomeres, or, reflect alterations in intracellular ionic composition (Powell *et al.* 2004). Disruption of the intracellular ionic environment would affect the contractile apparatus, sarcomere length and thus, the contractile response.

In both human hearts and animal models, maximal Na^+,K^+-ATPase pump activity has been shown to decrease during hypertrophy (Muller-Ehmsen *et al.* 2001; Paganelli *et al.* 2001). For the most part, this decrease is attributed to the reduced expression of the high affinity ouabain-sensitive catalytic α subunits or the common β regulatory unit of the pump (Muller-Ehmsen *et al.* 2001; Paganelli *et al.* 2001). In our present study, we found a 50% reduction in β1 protein in left ventricles of our adult IUGR females while β1 mRNA remains unaltered. Similar findings were reported in a rat model of renovascular hypertension with left ventricular cardiac hypertrophy where β1 protein decreased without any change in mRNA (Book *et al.* 1994). Diminished Na^+,K^+-ATPase pump activity would impact resting membrane potential, intracellular Na^+ concentration, as well as the activity of the Na^+–Ca^{2+} exchanger, leading to change in intracellular Ca^{2+} concentration and perturbations in the contractile response.

Changes in the expression of contractile elements are shown to occur in cardiac hypertrophy and may underlie diminished cardiomyocyte contractility as well. Myocardial contractile function improves with maturation and depends in part on developmental changes in the expression of components of the contractile apparatus (Fisher & Towbin, 1988). In the rat, the perinatal increase in myocardial contractile force occurs concomitant with changes in myosin isoforms (Fisher & Towbin, 1988). For example, in the rodent ventricle, there is a transition in the expression of MHC from the β to the α isoform (Mahdavi *et al.* 1987) as well as a segregation of specific MLCs with aMLC in the atria and vMLC in the ventricles (Swynghedauw, 1986). Hypertrophy is sometimes associated with a reversal of these phenomena in both humans (Nakao *et al.* 1992) and rats (Gupta & Gupta, 1997). In our IUGR adult females we found that aMLC-1 mRNA increased in the left ventricles while βMHC remained unchanged. Similar findings were reported in a rat model of pressure-overload where marked cardiac hypertrophy was observed without any change in βMHC mRNA (Wiesner *et al.* 1997). Up-regulation of MRLC-2 is associated with myocardial hypertrophy as well (Chien *et al.* 1991). However, for the most part, MRLC-2 expression is shown to increase in cell culture models of hypertrophy (Huang *et al.* 1997, 2002). In our animal model, MRLC-2 mRNA was unaltered.

Synthesis of ANP by ventricular myocytes is high during fetal and early neonatal periods decreasing markedly thereafter to reach adult levels (Chien *et al.* 1991). In myocardial hypertrophy, the ANP gene is re-expressed in the ventricle (Chien *et al.* 1991), making it a useful marker for cardiac hypertrophy. For example, a positive correlation between myocyte size and ANP immunoreactivity was shown in spontaneously hypertensive rats (SHRs) (Kaganovsky *et al.* 2001). A modulatory role has been proposed for ANP in cardiac hypertrophy as well (Gardner, 2003). In this regard, ANP produced by the ventricular myocytes and myofibroblasts could act, via its receptor, to suppress the activation of fetal genes associated with hypertrophy. In support of this, genetic disruption of the ANP receptor A in mice results in increased ANP expression, cardiac hypertrophy, and reactivation of fetal genes in the left cardiac ventricle (Holtwick *et al.* 2003). Although ANP gene expression increases in the IUGR rats, the receptor, which is normally present in the adult heart, may serve to inhibit reactivation of fetal genes such as β-MHC which is unaltered in our model.

We presently report that oxidative stress is implicated in cardiomyocyte hypertrophy in our female IUGR rats as well. It may underlie the decrease in Na^+,K^+-ATPase β1 expression in our model. In this regard, we show that the activity of aconitase, the mitochondrial TCA cycle enzyme that is most susceptible to inactivation by ROS (Vasquez-Vivar *et al.* 2000), decreased significantly in left ventricles of our IUGR female rats. In contrast, and as shown by others (Leary *et al.* 2002), citrate synthase activity is unaltered.

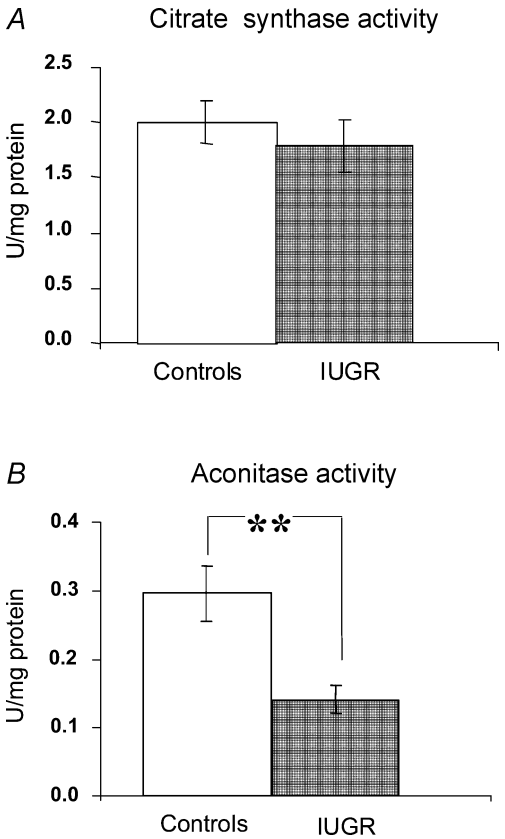

Figure 3. Activities for two TCA cycle enzymes in left cardiac ventricles of adult female control and IUGR rats
Activity of citrate synthase (*A*) and aconitase (*B*). $^{**}P \leq 0.01$ compared to controls.

In adult male SHRs treated with doxorubicin, Na^+,K^+-ATPase activity was shown to decrease in the left ventricles and the extent of cardiac hypertrophy correlated well with the increase in membrane lipid peroxidation (Torii *et al.* 1992). These changes in cardiac tissue were reversed by the antioxidant, α-tocopherol (Torii *et al.* 1992). Furthermore, in an *in vitro* model of cultured rat cardiomyocytes, non-toxic doses of ouabain were shown to suppress Na^+,K^+-ATPase activity, induce hypertrophic growth and increase transcription of marker genes for hypertrophy by mechanisms that implicate ROS generation (Huang *et al.* 1997; Xie *et al.* 1999). These experiments suggest a link between cardiac hypertrophy, the suppression of Na^+,K^+-ATPase activity and oxidative stress, and corroborate our present findings suggesting that ROS may be implicated in the hypertrophic response in our rat model as well.

In conclusion, we provide evidence for the fetal programming of cardiac remodelling in a rat model of IUGR. Programming, in the context of a prenatal Na^+ restriction model, appears to be limited to the female offspring and may reflect sex differences in the response to certain stressors. Although the underlying mechanisms that lead to the cardiomyocyte remodelling response in our animals warrant further study, our present data suggest that oxidative stress may be involved. The progression of initiation of hypertrophy to cardiopathy will be studied in older IUGR female rats and the use of antioxidants in preventing these remodelling changes in our animals will be investigated as well.

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Acknowledgements

We are grateful to Drs A. Tremblay and Rhoda Kenigsberg for their expert advice. This study was supported by grants from the Canadian Institutes of Health Research (MOP-37902). M.C.B., M.B and A.C received personal support from Fonds de la Recherche en Santé du Québec. J.C. holds a Canada Research Chair in Medical Genomics.